Turner Syndrome: Genotype & Phenotype

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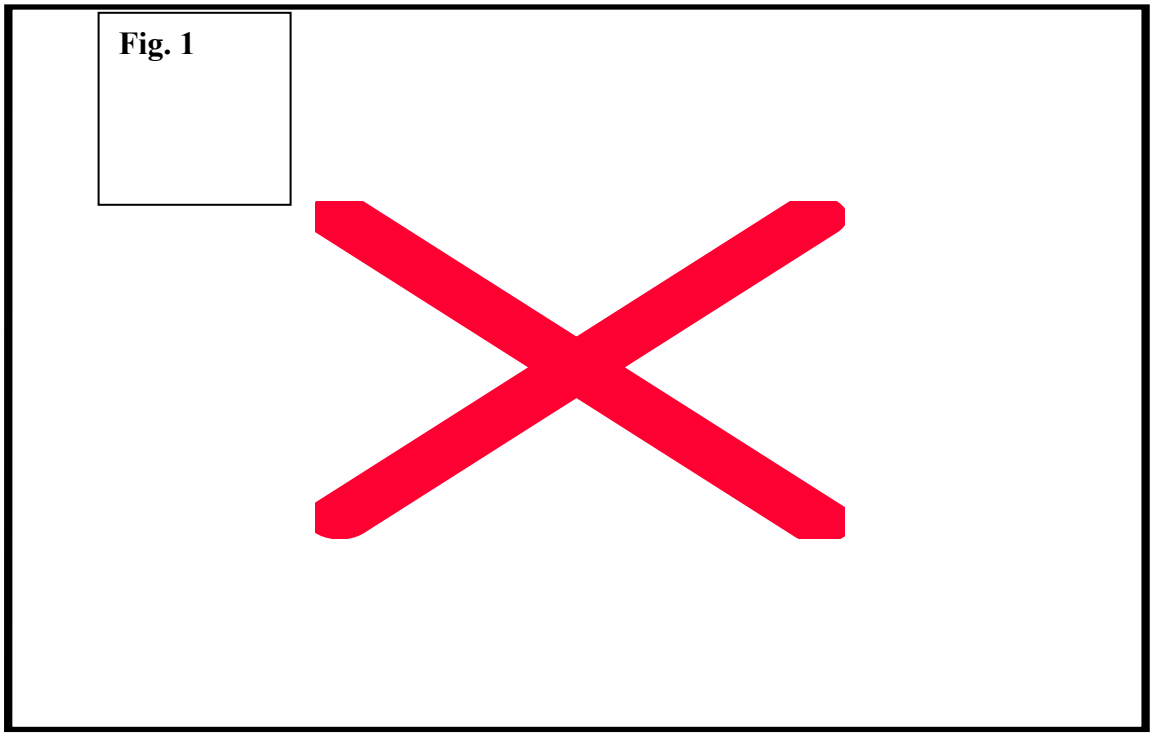
1. Precis

Turner syndrome (TS) is a sporadic disorder affecting ~ 1/2500 live female births. It is caused by the absence of all or significant parts of one sex-chromosome. Major developmental consequences include severe short stature, ovarian failure and distinctive cognitive and behavioral traits, with renal and cardiovascular defects affecting a minority. Adults with TS have excessive rates of osteoporosis, hypertension and diabetes mellitus and experience morbidity and mortality several-fold higher than the general population. Many of the problems of TS result from haplo-insufficiency for X-chromosome encoded genes, most of which remain unknown. Previous studies attempting to correlate genotype with phenotype in TS have been limited due to small numbers of subjects, limited genetic methodology and incomplete phenotypic characterization. This study aims to correlate TS phenotypes and genotypes using advanced clinical and genetic diagnostic methodologies, with the goal of identifying X-chromosome genes and epigenetic mechanisms causing the different features of TS. For TS subjects with a 45X genotype, the parental origin of the single normal X-chromosome will be traced to identify genomically imprinted features of the disorder. X chromosomal structural defects will be analyzed using high-resolution physical mapping in relation to emerging sequence data from the Human Genome Project. The elucidation of genetic mechanisms in TS will help improve the diagnosis and treatment of girls and women with this disorder and will further our understanding of gene dosage effects in general.

2. Introduction and rationale

Turner's syndrome (TS) is caused by sex chromosomal anomalies resulting in haplo-insufficiency for X-chromosome encoded genes [1]. The most common genotype is homogeneous 45 X (X0), which is thought to be due to non-disjunction or chromosome loss occurring during gametogenesis or embryogenesis [2, 3]. A recent study of 211 TS patients, employing sensitive cytogenetic and molecular diagnostic tools, found that XXq- was the next most common genotype, followed by XXr (ring X chromosome), XX/X0 mosaicism, XXp- and Xy- (Fig. 1). This study also confirmed that about 2/3 of the chromosomal defects in TS are paternal in origin [3]. Although large parts of one X-chromosome are normally inactivated in women, there are many X-chromosome genes for which biallelic expression appears to be essential for normal ovary, skeletal, lymphatic and nervous system development, and perhaps other important functions. Such genes are presumed to escape X-inactivation, and many are expected or known to have Y-chromosome homologues. For example, a gene required for normal skeletal development termed SHOX has been identified in the Xp pseudo-autosomal region [4, 5] and is implicated in the short stature and skeletal anomalies seen in TS.



About 25% of cases are diagnosed at birth on the basis of obvious defects due to lymphedema (cystic hygroma, webbed neck, low set ears) and skeletal abnormalities (cubitus valgus, short neck, short metacarpals, micrognathia). Additional cases are diagnosed later in life when they present for evaluation of short stature or pubertal delay, although a few girls with TS pubesce and menstruate for a number of years before experiencing ovarian failure [2].

Additional features of TS are summarized in Table I and include cardiovascular (mainly bicuspid aortic valve & aortic coarctation) and renal system defects (pelvocalyceal or renovascular anomolies, ectopic or horseshoe kidney). The reported incidence of the different features varies widely, with ascertainment bias being a major confounder. For example, the most clinically obvious defects (e.g., webbed neck) are related to lymphatic obstruction, which also causes aortic coarction, so this group is readily diagnosed. Another factor is the improved sensitivity of diagnosis using newer cytogenetic techniques and analysis of multiple cells for mosaicism, which have only been done in recent years. The most severe phenotype is associated with 45X. Since this was most readily diagnosed in the past, the more severe phenotypes are heavily represented in earlier series; Milder phenotypes are found in more recent series, using more sensitive diagnostic methods.

The identification of genes involved in TS ovarian dysgenesis has been elusive, with large regions of both arms of the X-chromosome associated with this phenotype (Fig. 2). The reasons for this apparent non-specificity remain unclear. It has been suggested that *any* major X-deletion may lead to oocyte death and ovarian atresia through meiotic misadventure rather than through haploid dosage of specific genes involved in oogenesis or ovarian development [6]. On the other hand, there have been specific X-chromosome loci and one identified gene (*DIA;* [7]) associated with karyotypically normal premature ovarian failure (POF), suggesting that specific ovarian function genes may be clustered in X-chromosome regions similar to the clustering of testis-determining and spermatogenesis genes on the Y-chromosome. The availability of a large population of karyotypically normal POF subjects in our other protocols may be of substantial value in clarifying these issues.

One issue of major medical concern and scientific interest is the fact that individuals with TS have twice the risk of developing Type 2 diabetes compared with the general population [8-10]. The metabolic features of TS have not previously been evaluated with respect to genotype-phenotype correlation. The TS metabolic syndrome includes insulin resistance, dyslipidemia and hypertension, similar to “Syndrome X”. Insulin resistance, manifested as glucose intolerance or overt type 2 diabetes, was found in 32% of over 300 TS subjects who had an oral glucose tolerance test (reviewed by Holl et al., [11]). Hypertension is reported in 15-40% [12-15]. Both insulin resistance and hypertension are found in prepubertal girls with TS, suggesting that these features are independent of the effects of ovarian hormone deficiency or therapeutic replacement. The reduction in insulin sensitivity was accounted for by reduced non-oxidative metabolism of glucose [16], suggesting that the principal lesion in TS is defective glucose uptake/utilization by muscle, as seen in typical adult type 2 diabetes. There is an apparent tendency toward obesity in TS [2, 13, 17], although body

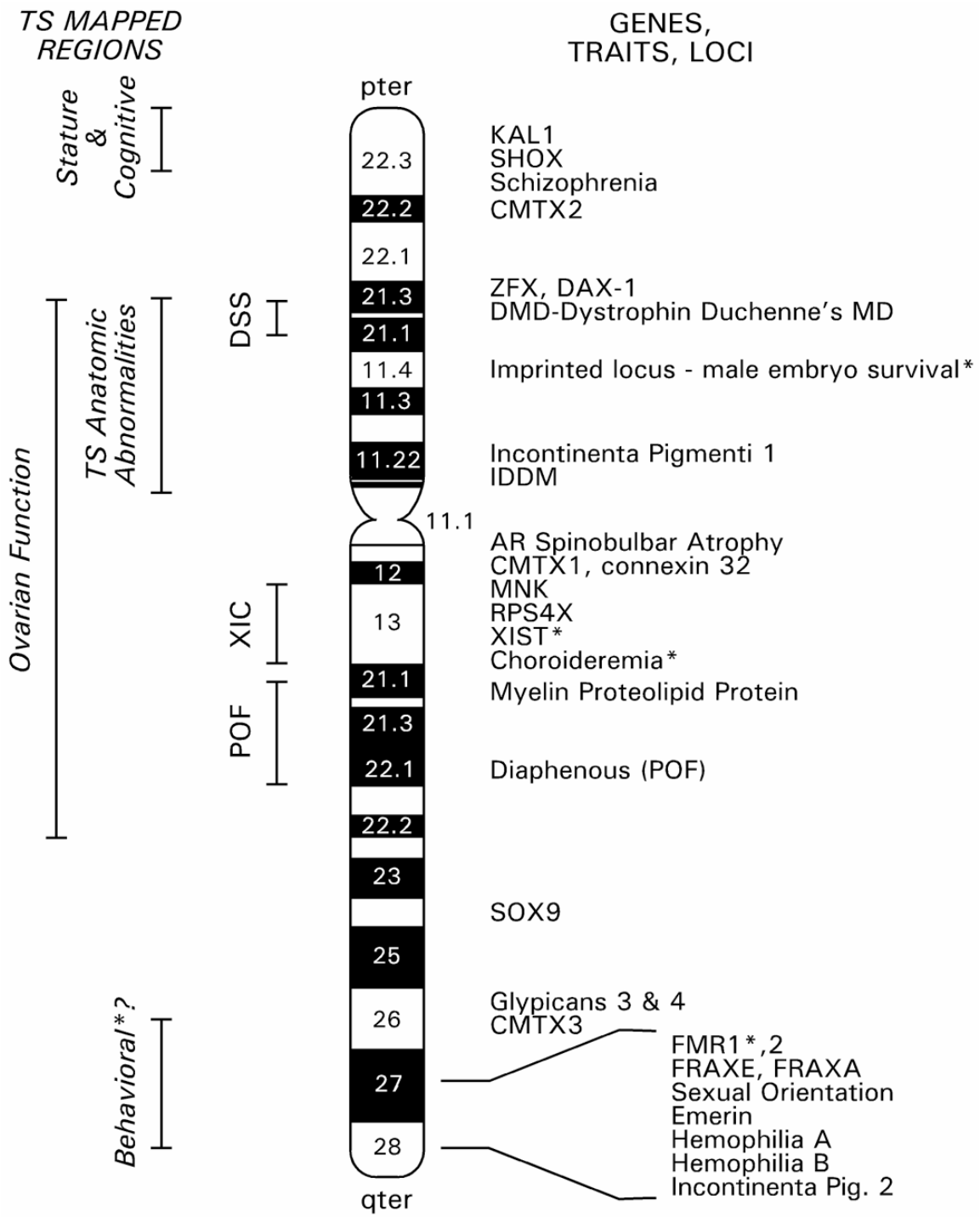


Fig. 2 Current mapping of X-chromosome loci and genes. Those regions specifically associated with TS are indicated on the right side of the figure. Loci marked by * are thought to be genomically imprinted.

composition not been well-studied in TS. Body mass index (weight normalized to height) is consistently reported to be increased, but this is not a good measure of obesity in TS given the altered body proportions due to the skeletal abnormalities. Using skin-fold thickness and ultrasound, two Japanese studies have found *reduced* subcutaneous fat in TS compared with age-matched controls [18, 19]. A European study employing bioelectrical impedance; however, reported a higher fat mass and a lower muscle mass in TS women [20].

Table I Clinical findings in TS

System	***Feature***	***Incidence***
Skeletal	Short stature	100%
	Scoliosis/kyphosis	?40%
	Osteoporosis**	50%
	Cubitus valgus	45%
	Short metacarpals	35%
	High arched palate	35%
Reproductive	1˚ gonadal failure	95%
	Infertility	98%
	Gonadoblastoma	3%
Lymphatic (obstruction)	Webbed neck	25%
	Low posterior hairline	40%
	Peripheral edema	20%
	Nail dysplasia	12%
Cardiovascular	Coarctation of the aorta*	5%
	Bicuspid AV*	15%
	HTN	25-40%
Orther embryogenic	Renal structural abnormalities	35%
	Mx pigmented nevi	25%
Neurological	Spatio-visual	70-80%
	Behavioral	70-80%
	Sensorineural hearing loss	30%
Endocrine	Hypothyroidism` (Hashimoto's)	35%
	Carbohydrate intolerance	30-40%

*Data from Prandstraller 99 [21] - this recent study is based on modern echocardiographic data and relatively free from preselection bias based on clinical presentation. It should be noted that coarctation of the aorta in TS is thought to be due to lymphatic obstruction in the preductal region rather than primary CVS developmental anomaly. ** The incidence of osteoporosis is based on a reported 50% fracture of typical

osteoporotic sites in adults with TS [9, 22]. The remainder of the data are based on Lippe, 1991 and Ogata, 1995 [2, 23].

From 25-40% of women with TS have high blood pressure [2, 14, 15]. Hypertension is attributed to congenital renal anatomic abnormalities or aortic coarctation in a few of these, but is designated as 'essential hypertension' in the majority. There has been little investigation of this aspect of TS and this will be the first systematic study of BP regulation in large numbers of subjects with TS. One possible explanation for the high incidence of 'essential' hypertension in TS could be subtle renal defects, given that gross abnormalities are seen in ~ one third of subjects with TS [2]. To investigate this potential etiology, the renin-angiotensin system will be evaluated by measurements of supine and upright plasma renin activity. An alternative explanation could be that hypertension in TS is related to insulin resistance, since both are reported to occur in about one third of patients with the disorder (although it is not known if the patients with hypertension are the same ones with insulin resistance). Hypertension is clearly associated with insulin resistance and hyperinsulinemia in the entity known as 'syndrome X,' and insulin per se has been implicated in arterial hypertrophy and non-compliance [24]. This potential association will be investigated by correlation of parameters of insulin resistance and blood pressure in subjects in the present study. Another potential cause of hypertension in TS could be a generalized connective tissue defect affecting bone, cardiac valve and vascular tissue.

The fact that the metabolic syndrome is reported in a consistent third of TS subjects across a large number of different studies suggests it might be related to paternal transmission, since one third of 45,X women have the paternal X-chromosome [3]. Parentally imprinted transmission of diabetic risk has been noted in non-TS diabetes [25], further supporting this possibility. Other significant problems seen in TS include hearing loss (both conductive and sensorineural), affecting about 50% in total, and autoimmune thyroid disease (mainly Hashimoto's thyroiditis), affecting about a third of TS patients. There is very little knowledge about the genetic basis for these features, which will also be addressed in the present study.

Girls or women who carry partial X- or Y-chromosomes in addition to their normal X may have some but not all of the typical clinical features of TS. Correlation of specific chromosomal abnormalities and phenotypes in such cases should provide clues as to the number and location of genes responsible for components of the TS phenotype. Attempts to correlate phenotype and genotype in TS have been hampered by the difficulty in collecting large numbers of subjects with informative deletions and the relatively crude cytogenetic and molecular techniques available in the past. For example, a relatively large study attempting phenotype:genotype correlations for the cardiovascular system included 136 patients [21], but only 29 had any kind of cardiac defect and 20 of these were homogeneous X0 and therefore uninformative. There were too few X deletions or rearrangements to yield clues as to loci responsible for cardiac defects. Current views of X-chromosome regions implicated in specific aspects of the TS phenotype are diagrammed in Fig. 2.

Recent advances in molecular cytogenetics and genomics hold potential for great new progress in elucidating the genes involved in the TS phenotype. Cytogenetic studies are now complemented and extended with high-resolution fluorescent *in situ* hybridization (FISH) and many X-chromosome specific molecular probes are now available. In addition, with the recent explosion in human genome sequence information (the X-chromosome is ~50% sequenced), we will be able to relate mapping data to specific gene sequences on the sex chromosomes. Also, there are now a fair number of candidate genes to analyze for involvement in TS phenotypes (e.g., *SHOX* in short stature, *DIA* in ovarian failure). Another new tack in our approach to TS has come about because of the recent demonstration that some X-chromosome genes are parentally imprinted. Genomic imprinting confers functional differences on parental genomes such that certain alleles are only expressed when they originate from one parent. Skuse et al [29] showed that 45X TS girls were significantly more likely to demonstrate cognitive and behavioral problems when they received their single normal X from their mother as opposed to their father. A proposed explanation for these findings is that a social skill-enhancing gene localized on the X-chromosome is silenced or imprinted on the maternal X, but actively expressed from the paternal allele. Another imprinted locus on Xp encodes a gene affecting survival of male embryos [26] and it has very recently been shown that ovarian failure associated with the fragile X pre-mutation (FMR, Xq27) occurs only with paternal transmission of the affected allele [27]. These findings suggest that other unknown TS genes might be subject to parental imprinting, and that analysis of the parental source of the normal X in 45,X patients with respect to the transmission of hypertension or insulin resistance may be very rewarding.

The present study will combine several strategies to enhance success in genotype:phenotype analysis of multiple features of TS. Firstly, highly sensitive molecular cytogenetic techniques will be employed to detect X-structural defects and the physical mapping will be translated to new sequence data available from the Human Genome Project. Secondly, parental DNA will be obtained from as many subjects as possible to elucidate the genomically imprinted transmission of different TS features. Finally, the clinical phenotypic analysis will be far more comprehensive than has historically been attempted. In addition to documentation of stature, skeletal anomalies and ovarian function, the most sensitive, "state of the art" clinical diagnostic tests will be employed to detect subtle cardiovascular and renal system defects, and to measure bone density, body composition, insulin sensitivity, cognitive functions and psychological profile.

Summary

This project aims to identify unknown genes involved in ovarian, skeletal and cognitive development and in the regulation of insulin sensitivity and blood pressure. Data from these clinical studies may also illuminate novel epigenetic mechanisms regulating gene expression at the chromosomal level. This comprehensive characterization of TS clinical phenotypes and correlation with specific genotypes will benefit girls and women with TS in terms of improved diagnosis and recognition of potential and existing medical problems, resulting in improved

preventative and therapeutic medical care. One of the most distressing aspects of TS for many patients has been the lack of medical and scientific information on the diagnosis and its implications [28]. This protocol will include genetic counseling and will provide TS participants with opportunities to ask questions and discuss their diagnosis and medical/social issues with the physicians, research nurses and genetic counselors involved in the study. Indeed, a specific goal of the study is the identification of major themes of interest and concern to people affected with TS and development of a counseling program that will effectively address these concerns (see Appendix C). The results of clinical and genetic studies will be provided in written form to study subjects and to their physicians. Care for pre-existing and newly found medical problems will be referred to a patient's personal physicians, or to qualified medical personnel in their area. TS patients older than 13 years of age may be eligible to participate in a companion TS hormone replacement study.

3. Objectives

1) To identify X-chromosome genes involved in the different phenotypic aspects of Turner's Syndrome

2) To elucidate regulation of X-chromosome genes by genomic imprinting

3) To identify major themes of interest and concern to women and girls affected with Turner syndrome and to define therapeutic aspects of genetic counseling through process analysis

Specific hypotheses we plan to test in this protocol:

- There are other genes involved in the short stature phenotype in addition to SHOX which may contribute to osteoporosis in addition to impaired long bone growth
- There are a number of discrete X-chromosome genes involved in ovarigenesis
- There is a type 2 diabetes susceptibility gene on the X-chromosome which may be imprinted
- There is a hypertension susceptibility gene on the X-chromosome which may be imprinted (and may be identical to the diabetes susceptibility gene— an X-chromosome syndrome X gene?)
- Specific behavioral traits as reported by Skuse et al [29] are parentally imprinted.
- There are X-chromosome gene(s) which determine specific cognitive abilities, apart from the effects of sex steroids on the brain

4. Study Design

Subjects will include phenotypic females 7 years of age and older with X chromosome defects, who will undergo a comprehensive, in depth phenotypic and genetic characterization, including:

- Anthropomorphic measurements
- Dysmorphology assessment
- Ovary function
- Skeletal development
- Bone mineral density
- Body composition
- Insulin sensitivity
- Lipid profile
- Blood pressure regulation
- Cardiovascular system anatomy & function
- Renal system anatomy & function
- Cognitive function (for subjects 14 –70 years)
- Psychological status (for subjects 14-70 years)
- Auditory function
- Thyroid function
- Molecular cytogenetic studies
- Parent of origin genetic analyses
- Genetic counseling evaluation

This evaluation needs to be done on an inpatient basis (4-5 days) since subjects need to be on a metabolic diet for the insulin sensitivity and BP studies. Subjects will provide blood cells for genetic analysis, and parental samples as well when possible. Cytogenetic and FISH studies will be used to define sex chromosomal abnormalities, including detection of cryptic mosaicism and presence of Y-fragments. Genotypes, including sex chromosome fragments or breaks, will be cloned in lymphocyte cell lines for further study. Sex chromosome breaks/deletions will be mapped to fine resolution using specific X-chromosome mapping probes (e.g., bacterial artificial chromosome clones), and physical data will be converged to emerging human X-chromosome sequence data from the Human Genome project to identify genes which when disrupted contribute to the TS phenotype. If the peripheral blood karyotype suggests mosaicism, a skin biopsy may be requested for further study of mosaicism in skin fibroblasts. The parental origin of the normal X-chromosome will be determined by comparison of X-polymorphisms in 45, X subjects with their parental sequences, and the frequency of specific TS clinical features analyzed with respect to parent-of-origin effects to elucidate aspects of genomic imprinting in X-chromosomal function.

Phenotypic and geneotypic data will be entered into a secured TS database, which is planned as a permanent resource to be maintained by NICHD for use by current and future investigators. All subjects participating in this study will be informed of the results of their evaluations in detail, and will be provided written summaries, as well as opportunities for discussion of clinical and genetic findings with study personnel. Many of the TS individuals participating in this study will be eligible for a

companion study, "Turner's syndrome: Hormone replacement therapy". The treatment protocol will be discussed with eligible subjects (which will include most individuals older than 13 years without major contra-indications to gonadal steroid treatment) during the inpatient NIH stay.

5. Subject selection

Inclusion criteria for TS subjects:
Phenotypic females > or = 7 years of age
Evidence of X-chromosomal abnormality
Euthyroid status documented by normal TSH obtained prior to admission

Exclusion criteria for TS subjects:
Co-existing autosomal defects
Pregnancy
Growth hormone or androgen treatment within 6 months

Inclusion criteria for the parents of TS subjects (for DNA only):
Biological parent of a TS subject
Willingness to participate

Exclusion criteria for the parents of TS subjects (for DNA only):
None

6. Study procedures

Screening:
Candidates with a karyotype demonstrating X-chromosome monosomy, breaks or deletions (in the absence of other major chromosomal defects) will be scheduled for admission to the NIH clinical center. Consent forms and information about the study will be mailed to candidates, and study investigators will discuss the study with candidates by phone prior to admission. Candidates with a clinical suspicion of TS (e.g., short stature and premature menopause) will be screened with a karyotype performed at NIH as an outpatient. If the peripheral blood karyotype is normal in such an individual, cells may be obtained from skin biopsy for cytogenetic analysis, since monosomic cell lines may selectively die out in the bone marrow [30, 31].
Evidence of euthyroid status will be required prior to admission, since many patients have hypothyroidism, which untreated would confound metabolic and cardiovascular evaluations. The study will be explained to candidates interested in participating. All study procedures, risks, and benefits will be discussed with candidates. Consents or assents will be obtained, and subjects will be informed of their right to withdraw from the study at any time. **Subjects will be asked to discontinue estrogens, GH, lipid-lowering and anti-osteoporosis medications for 2 weeks prior to admission.**

6.1 Methods for achieving study objective 1

Upon admission to the NIH CC, subjects will be placed on a metabolic diet consisting of 25% fat, 50% carbohydrate and 25% protein with 4 g sodium.

Phenotypic characterization (see schedule of testing in Appendix A)

a) Anthropomorphic measurements including sitting and standing heights, weight, hip & waist measurements
b) Dysmorphology assessment- description of neck webbing, ear placement, hairline, palate, palpebral fissures, metacarpals and -tarsals & cubitus valgus with photo documentation
c) Biochemical assessment including blood chemistries, thyroid and liver function tests
d) Ovary function- history of menarche and menses, Tanner staging, and measurement of estradiol, testosterone, free testosterone, androstendione, SHBG, LH, and FSH, ovary ultrasound. Subjects < 18 yrs will have a trans-abdominal US. Older subjects who are thought by the investigators to have inadequate sexual maturation and/or are not sexually active will have trans-abdominal US. Older subjects who have sexually matured and are sexually active will have a vaginal US.
e) Skeletal development will be assessed by xray of the wrists, with evaluation of the Madelung deformity and bone age. All adults will be screened for the presence of scoliosis and/or kyphosis by AP and lateral films of the spine. Children will not have these studies unless clinically-indicated.
f) Bone mineral density (BMD) measured at the lumbar spine, wrist & hip using DXA. Because of the small size of subjects with TS, DXA BMD data will have to undergo volumetric correction, providing an indirect reflection of true mineral content. Therefore, adults (18 years and older) will also undergo a quantitative CT (qCT) analysis of BMD at L1-L2, providing a direct measure of bone mineralization. Heel BMD will be assessed by ultrasound and correlated with qCT and DXA results. Serum and urine markers of bone turnover will also be measured (Appendix A).
g) Radius xrays for evaluation of radial deformity characteristic of TS ('Madelung')
h) Bone age xray for girls who have not yet entered puberty (included in radius xrays)
i) Body composition is examined using DXA for whole body fat, muscle and bone, qCT of adults (> or =18 yrs) for visceral fat (this is done on the same study for vertebral BMD), and MRI for children (< 18 yrs) for visceral fat. The children will not get the quantitative CT because we want to avoid exposing children to radiation wherever possible.
j) Insulin sensitivity will be evaluated by fasting insulin and glucose levels and glucose and insulin responses to an oral glucose tolerance test as described by Reaven and co-workers in Diabetes Care 23:171-175, 2000. In brief, we will measure fasting glucose and insulin and the total integrated insulin and glucose response to a 75 g oral glucose challenge.
k) Lipid profile from a morning fasting blood sample: HDL & LDL cholesterols, dense core LDL, triglycerides, apolipoproteins A1 & B, Lipoprotein (a)
l) Cardiovascular evaluation: ECG; echocardiography including Doppler studies and color flow mapping using a 2.5 MHz transducer. The evaluation will include standard M-mode

measurements and 2-dimensional evaluation from all standard planes. Flow velocities across the aortic, mitral, bicuspid and pulmonic valves as well as the ascending and descending aorta will be obtained using pulsed and continuous Doppler modalities. In addition, the base of the heart and aortic root will be studied by MRI, to detect coarctation and possible degeneration suggesting risk for dissection. All studies will be recorded electronically and reviewed by a cardiologist blinded to the genotype.

m) Blood pressure: measurements q 8 h while subjects are on a 4 g sodium metabolic diet during the 4-5 day inpatient stay. A portable arm cuff unit will be used for ambulatory 12-24º BP recording to be done while at the CC. Plasma renin activity (PRA) will be measured on the 4th inpatient day, in the supine (early a.m.) and upright positions. The renin-angiotensin system is targeted because of the likelihood of renovascular disease in these patients. Renal system anatomy will be assessed by ultrasound and renal function by urinalysis, 24h urine protein excretion and creatinine clearance

n) Metabolic panel- these tests of serum factors evaluate cardiometabolic or syndrome X features thought to contribute to the development of cardiovascular disease

o) Cognitive function will be evaluated by a battery of standard tests measuring visual/motor/perceptual and verbal skills for subjects 14-70 yrs (Appendix B)

p) Psycho-social evaluation will consist of a battery of standard tests measuring self-image, quality of life, mood and sexual function for subjects 14-70 yrs (Appendix B)

q) Specific issues of concern to women and girls related to the diagnosis of Turner syndrome and genetic counseling needs will be evaluated as described in Appendix C

r) Audiology testing and CT scan of the inner ear. The origin of hearing loss in Turner syndrome will be investigated by our collaborator, Dr Andrew Griffith. Sensorineural hearing loss in Turner syndrome may be caused by loss of X-linked gene/s directly involved in aural neural function, by the ototoxic effects of chronic otitis media or by abnormal morphologic development of the inner ear. The possibility of inner ear morphogenetic abnormalities is suggested by the many skeletal abnormalities observed in Turner syndrome. Possible inner ear dysmorphology may be directly addressed via high-resolution CT imaging of the temporal bone structures. Inner ear malformations are observed in only a small percentage of patients with sensorineural hearing loss caused by either genetic or nongenetic etiologies, and thus may serve as a specific auditory phenotypic feature to define the etiology(ies) of sensorineural hearing loss in Turner syndrome, and to eventually identify potential causative genes and mechanisms. Thus, to elucidate the cause of the hearing loss in Turner syndrome, we propose to study the anatomy of the internal auditory canals in 30 adults with Turner syndrome using CT scan in 1 mm slices at a 1:1 pitch and 120 kV, 250 mA. Children will not be studied because of the additional radiation.

Normative data for most of the above parameters are abundant. In addition, we have data on most of the above tests for large numbers of karyotypically normal women with premature ovarian failure which can be used to isolate the secondary effects of ovarian insufficiency from X-chromosome genetic defects. The results of these tests will be entered into a TS database, which will facilitate the investigation of potential linkage between traits (e.g., insulin resistance

and hypertension, insulin resistance and low birth weight, hearing loss and social skills), as well as the linkage of different specific clinical features and specific genetic lesions.

Genetic analyses

Routine G-banding karyotyping will be done on 50 metaphase lymphocytes for all subjects. Abnormal X-chromosomes will be subjected to molecular cytogenetic analysis. Fluorescence in situ hybridization (FISH) with an X centromere probe (DXZ1) and a whole X chromosome painting probe will be performed to characterize aberrant X chromosomes and to look for mosaicism. At least 100 peripheral blood leukocyte metaphases will be examined to exclude low-level mosaicism with 99% confidence [32]. If equivocal low-level (<5%) mosaicism is detected, skin fibroblasts will be obtained for additional cytogenetic and FISH analyses.

Lymphocyte cell lines from patients with X-chromosome breaks or deletions or inversions will be immortalized for further molecular analyses. These samples will be coded so that the subject's identity cannot be determined from the cell lines. A panel of single-copy X-specific clones whose precise map locations are known (Table 2) will be used as FISH probes to characterize rearrangements. Each hybridization will include the X centromere probe (DXZ1), detected using a second fluorophore, which unambiguously identifies the X chromosomes and provides an internal control for hybridization and for random chromosome loss.

Table 2.

Locus	Δ from Xp tel, Mb	Probe type
DXYS14	0	cosmid
SHOX	0.5	cosmid
DXYS147	2	PAC
ARSD	3	PAC
DXS118E	5.8	PAC
DXS7470 (KAL)	9	BAC
PDHA1	20	cosmid
DXS274	23	cosmid
GK	33	cosmid
DXS1110	40	BAC
SYN1	46	cosmid
SYP	50	cosmid
DXS423E	54	cosmid
ZXDB	58	cosmid
DXZ1	59-62	plasmid
DXS339	67	cosmid
XIST	74	cosmid
POU3F4	82	cosmid
DXYS1	90	BAC

BTK	100	P1
DXS287	110	BAC
DXS1001	120	BAC
HPRT	132	BAC
DXS984	140	BAC
FMR1	151	cosmid
PAR2	160	cosmid

Breakpoints from subjects whose deletions may potentially narrow critical regions for specific Turner phenotypes will be fine-mapped to at least 1 Mb resolution, either by FISH using additional mapped large-insert clones available via the Human Genome Project (e.g., The Sanger Center, Roswell Park Cancer Institute), or if parental DNAs are available, by analysis of polymorphic microsatellite markers as described [33].

When a locus has been mapped to a small (< 1Mb) critical region, fine grain resolution using BAC probes associated with X-chromosome sequence analysis will be employed to connect the physical map of the critical region with the sequence maps of the X chromosome (Locus link contigs). Sequence data from the Human Genome Project will provide a set of candidate genes within the critical region for further biological study.

6.2 Methods for achieving study objective 2

Parental DNA will be obtained from blood samples or where this is not practical, by oral mucosal brushes which can be sent via mail. Samples will be sent to the laboratory of Dr. Andrew Zinn at UT Southwestern Medical School in Dallas, Texas. In the case of 45,X subjects, the parental origin of the single X will be determined by comparing proband and parental DNA polymorphisms using PCR amplification of alleles of the following microsatellite loci:

Locus	Assay	GDB No.	Location
AR	AR.PCR1	185508	Xq12
DXS981	STRX1	187674	Xq12
DXS101	——	207667	Xq22
HPRTB	HPRT.PCR3	185506	Xq26.1

Each of these markers is at least 80% informative; therefore, at least one marker is expected to be informative in 99.8 % $(1-(.2)^4)$ of individuals. In the less frequent instances of partial deletions, the parental origin of the normal X chromosome will be determined by genotyping polymorphic markers located within the deleted region (determined by FISH), as described [33]. Oligonucleotide primers specific for each locus are available from Research Genetics. Phenotypic variation between monosomic subjects with a maternal X compared with those with a paternal X will support the presence of an imprinted genetic locus. In the case where only one parent is available: if the available parental sample differs in these markers from the proband, the assumption is made that the proband obtained the X chromosome from the other parent. If the single parental

sample matches the proband, additional studies using up to 10 different primer sets will be performed to determine with up to 98% certainty that this parent contributed the proband's X-chromosome. As genome sequencing and elucidation of the rate of individual variations is rapidly progressing, it may be possible in the near future to determine with almost 100% certainty the source of the proband's X-chromosome even with only one parental genetic material

6.3 Blood Sample Collection and Analysis

Appendix A specifies the schedule of testing during the study. Blood samples will be collected according to NIH guidelines. Serum FSH, LH, estradiol, testosterone, free testosterone, SHBG, DHT, DHEAS, androstendione, serum bone specific alkaline phosphatase, PINP, collagen cross-laps, osteocalcin, and urine NTX will be analyzed at Endocrine Sciences. The remaining blood samples will be analyzed at the NIH Clinical Center.

7. Human Subject Protections

a. Rationale for research subject selection:
Turner syndrome is a sporadic disorder affecting ~ 1/2500 live female births, caused by the absence of all or significant parts of one sex chromosome. Major developmental consequences include severe short stature, ovarian failure and distinctive cognitive and behavioral traits, with renal and cardiovascular defects affecting a minority. Adults with TS have excessive rates of osteoporosis, hypertension and diabetes mellitus and experience morbidity and mortality several-fold higher than the general population. Many of the problems of TS result from haplo-insufficiency for X-chromosome encoded genes, most of which remain unknown. Previous studies attempting to correlate genotype with phenotype in TS have been limited due to small numbers of subjects, limited genetic methodology and incomplete phenotypic characterization. This study aims to correlate TS phenotypes and genotypes using advanced clinical and genetic diagnostic methodologies, with the goal of identifying X-chromosome genes and epigenetic mechanisms causing the different features of TS. For TS subjects with a 45X genotype, the parental origin of the single normal X-chromosome will be traced to identify genomically imprinted features of the disorder. X-chromosomal structural defects will be analyzed using high-resolution physical mapping in relation to emerging sequence data from the Human Genome Project. The elucidation of genetic mechanisms in TS will help improve the diagnosis and treatment of girls and women with this disorder and will further our understanding of gene dosage effects in general. TS affects all racial and ethnic groups equally, so subjects will be recruited from all races/ethnic groups. Research subjects who are cognitively or physically impaired are included in the study if they have a parent or legal guardian empowered to consent for them, if needed, and assist them in participating to the extent that they are able. Since TS affects only females, males will not be recruited for this study, unless they are one of the biological parents of a TS subject. Biological parents of TS subjects are included only for DNA testing.

b. Strategies / procedures for recruitment:
Patients for this study will be recruited by advertising in medical journals, press releases, letters to physicians and TS contact groups, and by the NIH home page. Special attention will directed at Medical facilities known to serve large minority populations to help us enroll substantial numbers of African American and Asian subjects. All advertisements and letters will be reviewed by the IRB. In addition, subjects participating in current NICHD TS protocols 87-CH-0152, 90-CH-123 and 93-CH-0054 will be invited to participate. In addition, patients from our short stature and premature ovarian failure clinics routinely undergo cytogenetic screening and will be referred to this TS genetic study when X-chromosome abnormalities are found.

c. Justification for exclusions:

Men are excluded because subjects with a missing or fragmented 2nd X-chromosome are women. Subjects younger than 7 are excluded because they are too young to cooperate in the various study procedures and because normative data are scarce for this age group.

d. Benefits

By participating in this study, girls and women with TS will learn more about their disorder's diagnosis, clinical features and genetic causes. They will benefit from a comprehensive medical evaluation and learning about which of the many features of TS they may have. For example, since risk for multiple bone fractures is extraordinarily high in TS, it may be of considerable benefit to the individual to learn if her BMD is so low as to place her at risk, since there are a number of strategies which may improve bone strength and prevent fracture (e.g., diet, exercise, medications). The same rationale applies to the discovery of subclinical cardiac valvular defects, which may require anti-bacterial prophylaxis, and identification of impaired glucose tolerance, which may allow corrective steps to prevent the development of overt diabetes. Finding out about such issues may be viewed as a benefit of participation in this study.

One of the major concerns of women affected with TS has been their lack of knowledge about the genetic and clinical aspects of the disorder, and inability to find clinicians able to provide essential information and discuss issues related to the diagnosis [28]. Thus, participating in this study may help alleviate these concerns and be viewed as a benefit to their participation in research.

e. Risks/Discomforts:

Blood collection: The vein and venipuncture site may become inflamed and tender, and some leakage of blood could occur giving the appearance of a bruise. There are remote risks of fainting or of developing a local infection. The total blood volume for the research tests is 140 ml, which is within the NIH guidelines of 450 ml for adults and 7cc/kg for children > 20 kgs in each 6-week period. The amount of blood drawn will be limited for those children who weigh less than 20 kg to ensure that they do not exceed the NIH guidelines for blood volume. See Table II in Appendix A.

Radiation exposure:

Adults: A pregnancy test will be obtained from menstruating women before these procedures, which will not be performed if the test is positive. Dual energy x-ray absorptiometry (DXA): The radiation exposure from each bone mineral determination is 0.010 rem to the skin of the upper femur, 0.010 rem to the skin of the antero-posterior lumbar area, 0.035 rem to the skin of the lateral lumbar spine area, and 0.005 rem to the skin of the wrist. Whole body DXA-scanning for body composition provides a maximum total radiation exposure at any site of about 0.001 rem for the Hologic QDR-4500A. The lumbar vertebral quantitative CT for BMD involves about 0.825 rem to L1-L2. Both wrists will be x-rayed (AP & lateral) to document possible Madelung's deformity. The

radiation exposure from these x-rays will be 40 mrem. The total absorbed radiation dose received from this study is within the NIH Radiation Safety Guidelines for adult research subjects, 3 rem to any tissue in a 13-week period and 5 rem in one year.
Children: Bone age x-ray (for subjects <18 yrs) radiation to the left wrist/hand is 20 millirem, but this will be included in the wrist x-rays. Both wrists will be x-rayed (AP & lateral) to document possible Madelung's deformity, so the total will be 40 mrem. Dual energy x-ray absorptiometry (DXA): The radiation exposure from each bone mineral determination is 0.010 rem to the skin of the upper femur, 0.010 rem to the skin of the antero-posterior lumbar area, 0.035 rem to the skin of the lateral lumbar spine area, and 0.005 rem to the skin of the wrist. Whole body DXA-scanning for body composition provides a maximum total radiation exposure at any site of about 0.001 millirem for the Hololgic QDR-4500A. Children will not undergo the qCT. The total absorbed radiation dose received from this study is within the NIH Radiation Safety Guidelines for children, 0.3 rem to any tissue in a 13-week period and 0.5 rem in one year.

Other discomforts:
A few subjects with equivocal mosaicism in peripheral blood (estimated to be ~5/400) may be asked to undergo a skin biopsy. This will be done under local anesthesia and sterile conditions. A very small incision is made, and a small amount of dermal skin is removed. The site is closed with steri-strips. There is a small risk of minor bleeding, bruising or infection associated with the procedure. Pain at the site is very rare.

Some patients experience MRI claustrophobia or are unable to hold still for the required time. If this is the case, mild sedation with an anxiolytic may be used, if there are no contraindications in adults. For children, sedation will not be used unless the study has been unsuccessful in its absence. If sedation thus appears required, a physician trained in the conscious sedation of children will discuss the issue with the child and parents and obtain a specific consent covering the procedure.

Subjects will have an IV inserted into their arm vein for the oral glucose tolerance test (OGTT). The catheter will remain in the vein for several hours while the test is taking place. Topical anesthetic will be used to insert the line for children and for adults who wish it. There is a possibility of bruising, bleeding or infection at the IV insertion site, which will be closely monitored.

The psychological and neurocognitive testing and the genetic counseling interview each take from 1-2 hrs and thus may be inconvenient or tiring for some subjects. Some questions or topics may potentially cause embarrassment or emotional distress to some subjects. The investigators have been trained in recognizing and being sensitive to such discomfort and will take measures designed to make study subjects as comfortable as possible with the testing. The tapes of genetic counseling interviews will be coded by numbers so that the subject's identity is protected. The tapes will be stored in a secure,

locked cabinet accessible only to study investigators, and will be destroyed as soon as they have been analyzed.

Adverse occurrences:
We don't expect much in the way of adverse occurrences with this protocol, which involves no investigational drugs or invasive procedures. All subjects, however, are to be instructed to report any and all adverse events possibly related to protocol participation to one of the study investigators, in particular Lori Hanton, RN. Study investigators will report such occurrences to the PI, who takes responsibility for reporting *serious* events to the CD and IRB as soon as possible, and other occurrences in the annual review, following the Interim Guidelines issued by the DDIR.

Treatment/Compensation
No compensation will be offered to study participants. Travel will be paid for subjects who meet NICHD guidelines for such payment. Treatment will not routinely be provided for conditions identified during the NIH evaluation. However, abnormal results will communicated to participants and their designated physicians.

Issues related to pediatric subjects
This study involves only minimal risk for subjects <18 years of age— physical exams, anthropometric measurements, blood draws, DXA, MRI and ultrasound imaging. The radiation is minimal and confined mainly to the lower arms for documenting the potential for further growth (bone age) and the presence of Madelung's deformity. The protocol takes into account psychological as well as physical discomfort, and provision has been made for soliciting the assent of children and the permission of parents.

Consent and Assent Processes
Consent forms and information about the study will be mailed to candidates, and study investigators will discuss the study with candidates by phone prior to admission. All study procedures, risks, and benefits will be discussed with candidates. Consents and assents (for subjects <18 years) will be obtained, and subjects will be informed of their right to withdraw from the study at any time. Study subjects will be asked for permission to have their parents contacted about contributing DNA for the study, and if agreed, the parents will be contacted directly by the investigators and consents for participation (contribution of DNA) obtained.

Confidentiality: Results of the research studies under this protocol will be coded numerically and entered into a secure database so the study subjects cannot be identified except by the principal investigator. A certificate of confidentiality will be obtained to protect participants from unwanted intrusions into their research files.

8. Data management and analysis

The clinical and genetic data from this study will be entered into a secure TS database which will facilitate analysis of linkages between specific traits and between traits and specific genotypes. To our knowledge, this is the first and only project to attempt the creation of such an important resource for this disorder.

To obtain enough subjects to link specific traits such as hypertension, cardiac valve lesions or insulin resistance with X-chromosome genetic defects, we estimate a need for at least 400 enrollees. This projection is based on the reported incidence of about 30-40% for these traits, and genetic diagnoses of informative X-chromosome lesions of ~20% from more recent studies with relatively good genetic screens [3, 21]. We expect that with 'state of the art' genetic testing we will be able to improve the ratio of informative X-chromosome lesions. Thus, if 120 patients (30% x 400) have a trait of interest, ~ 24 of these will have X-chromosome lesions that may provide clues as to the genes involved in the phenotype. Despite the low yield, this number of subjects with informative phenotype-genotype data represent a relative wealth of information in the field of genetics.

Given the more prevalent 45, X monosomy genotype, we expect more abundant results from the parent of origin analysis with respect to traits such as insulin resistance. If as suggested by the literature, 50% of the patients are relatively pure monosomy X, then we will have 200 such genotypes. If as reported, 32% of these demonstrate insulin resistance by OGTT, we will have ~60 patients with insulin resistance and pure 45, X. If we can obtain parental DNA from 2/3 of these patients, we will have an *n* of 40 for analysis of genomic imprinting for the insulin resistant phenotype.

After accrual of 100 patients, we plan to assess the genetic data and may revise our predicted recruitment needs.

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Appendix A: Schedule of testing for TS Genotype-phenotype

TS: Genotype-phenotype EVALUATION	BLOOD VOL
Sign consent	
Physical exam	
UA/Urine pregnancy test ***	
CBC	3 ml
Chem & mineral panel, LFTs	3 ml
Bone markers	10 ml
Sex hormones	10 ml
Metabolic panel, TFTs	27 ml
OGTT	20 ml
Fasting lipids	8 ml
U & S PRA	6 ml
24˚ urine albumin, Cr	
Body & BMD DXA	
Lumbar qCT*	
Abdominal MRI**	
Radius x-rays	
Bone age x-ray**	
Spine AP & Lateral*	
Ear CT*	
Renal, Heel, & Pelvic U/S	
Cardiac Echo, MRI, EKG	
Audiology/ENT	
24˚ BP	
Neurocognitive tests	
Genetic counseling	
Psychosocial testing	
Research blood sample	20 ml
Genetic studies/Karyotype	20 ml
Parental DNA	

*adults only; ** children (<18) only; ***have had spontaneous menses

Metabolic panel: C-RP, HA1C; Amylin, leptin, IGF1, IGFBP1, IGF2, PAI-1, ACE
Lipids: HDL & LDL, TG's, apolipoprotein A1 & B and Lp(a); dense core LDL (NMR Lipoprofile)
Sex hormones: Total and free T, E2, androstendione, DHEAS, DHT, FSH, LH, SHBG
Bone Markers: PINP, serum cross-laps, osteocalcin, BSAP, urinary NTX

Appendix B

Neurocognitive Evaluation of Women with TS (Judith Ross, M.D.)

The neurocognitive tests listed below have been chosen in order to cover those cognitive and behavioral domains that have been shown to be sensitive to treatment with sex hormones. We will also take the opportunity to administer additional tests to further characterize our subjects at the time of evaluation, since this will entail minimal additional time or cost. In order to minimize any sequence effects of the test battery on test performance, the particular first test administered in a standard sequence of tests will be varied. We will not administer the battery in a completely randomized sequence, because time requirements would increase as would the likelihood of omitting or duplicating tests. All of the evaluations will be performed by an experienced Master's level psychometrician in conjunction with and under the supervision of our neuropsychologist, Dr. Michele Mazzocco, who has doctoral and postdoctoral training in pediatric neuropsychology. The testing duration at each session will be less than four hours. Socioeconomic Status (SES): SES will be derived from the Hollingshead 2-Factor Index of Social Status based on education and occupation (Hollingshead et al., 1958).

General cognitive
Wechsler Adult Intelligence Scale -III (WAIS-III) (Wechsler, 1997). The WAIS-III is a widely used measure of intellectual functioning for adults between the ages of 16 and 89. The instrument has been standardized on 2,450 adults with equal representation of geographic region, race, socioeconomic status, and gender. Reliability data cited in the manual indicates excellent reliability for the Verbal, Performance, and Full Scale IQs at 0.97, 0.93, and 0.98, respectively. Test-retest reliability over a 2-12 week period was 0.96, 0.90, and 0.96 for VIQ, PIQ, and FSIQ, respectively.

SPATIAL COGNITION

Spatial perception
(1) Kaufman Gestalt Closure (Kaufman et al., 1994). This test is a measure of perceptual closure or the ability to identify incomplete figures. Impaired performance on this spatial, non-motor task is associated with right hemisphere dysfunction. The subject is presented with fragmented depictions of common objects and is asked to identify the whole by perceptually integrating the fragments into a whole percept. Normative data is standardized from 1,983 subjects, ages 11 to 85 years as well as from 60 female adults from our own normative sample. Test-retest reliability was 0.76. Split-half reliability ranged from 0.73 to 0.87.
(2) Test of Facial Recognition (Benton et al., 1994). This task has a substantial visual-perceptual processing component and is also sensitive to right parietal dysfunction. It requires the subject to match a face with a series of target faces. It assesses visual discrimination of the complex visual array associated with facial features. It will be administered and scored following the standard protocol. The test plateaus at approximately age 14; however, weakly lateralized adults also have relatively impaired performance. The response time will be recorded in order to increase the sensitivity for the >14 year old age group. We have collected a sample of 60 adult women, and results differed significantly between adults with Turner syndrome and controls.
(3) WAIS-III Picture Completion. This scale assesses spatial perception and closure, as well as the subject's ability to discriminate between essential and nonessential detail.
(4) The Visual Object and Space Perception Battery (VOSP, Warrington et al, 1991). This battery provides an assessment of object and space perception ability. Each test focuses on one component of visual perception while minimizing the involvement of other cognitive abilities. The tests used in our research include Silhouettes, Progressive Silhouettes, Number Location, and Cube Analysis. The Silhouettes test measures the subject’s ability to perceive common objects photographed from unusual angles. Progressive Silhouettes, is very similar to Silhouettes, except now a series of silhouettes of the same object are presented. These silhouettes vary the angle of view from 90 degrees rotation to 0 degrees lateral rotation. The Number Location test assesses simple dot position discrimination.. The Cube Analysis test provides a measure of the perception of complex spatial relationships. Norms are available from 350 adults, ages 20-69 years as well as our own group of 60 women controls.

Spatial construction

(1) Rey-Osterrieth Complex Figure (Waber et al., 1985). The subject's organizational style, perceptual accuracy, and motor planning will be assessed from the direct copying portion of the task. Norms are available on adults, ages 16-60 as well as 60 of our own adult subjects. The organizational score is calculated according to the method of Waber and Holmes (Waber et al., 1985).

(2) Developmental Test of Visual-Motor Integration (Beery, 1989). This task evaluates the subject's ability to rapidly and accurately reproduce a series of simple figures after practicing conceptualizing and reproducing each figure. The function of this task is to measure visual fine-motor ability while limiting potential effects of conceptual differences and prior learning. It will be administered and scored following the usual protocol. Standard scores are available based on a sample of 5824 children, ages 2-17 years as well as our own normative sample of 60 female adult controls. Test-retest and interrater reliabilities are moderate to good, ranging from 0.63 to 0.92. This test has been found to discriminate between adult Turner subjects and controls in our own sample.

(3) Block design. This WAIS-III subscale measures abstract, nonverbal reasoning and assesses spatial construction ability.

Spatial orientation

(1) Mental Rotations Test (Kimura, 1992). This test measures spatial transforming ability, using two irreducibly asymmetric, mirror-image, three-dimensional forms. The children are presented with sequential pictures of one of the two forms at various angular orientations and must match the picture to the identical form. The dependent variables are the number of mental rotations problems correctly solved (out of 32) and the time required. We have acquired normative data from 200 children and adults.

Spatial memory

(1) Rey-Osterrieth Complex Figure-Recall (Akshoomoff et al, 1995). This aspect of spatial memory has been studied in children and adults and has been normed for 100 women in that age range.

(2) Warrington Memory Test (Faces) (Warrington, 1984). This test evaluates nonverbal recognition memory for faces. Norms are available from our own sample of from 60 female adults as well as 310 adults, ages 18-70.

(3) The Wechsler memory Scale-III (WMS-III) (Wechsler, 1997). The WMS-III is an instrument designed to appraise major dimensions of memory functioning in adolescents and adults. It is comprised of a series of brief subtests each measuring a different facet of memory. The functions assessed include memory for verbal and figural stimuli, meaningful and abstract material, and delayed as well as immediate memory. This instrument was normed on 1,250 subjects ranging in age from 16 to 89 years. The test-retest stability coefficients ranged from 0.74-0.93.

ATTENTION/IMPULSIVITY

(1) WAIS-III Working Memory Index (Wechsler, 1997). This factor is comprised of 3 subtests (arithmetic, letter-number sequencing, and digit span) from the WAIS-III. This measure is based on factor analysis studies of the WAIS-III and correlates significantly with other measures of attention.

(2) Test of Variables of Attention (TOVA) (McCarney et al., 1990). This test is a visual continuous performance test designed for the diagnosis and monitoring of children and adults with attention deficit disorders. It is a non-language based, 22 minute, computerized test that requires no right-left discrimination and has negligible practice effects. Norms have been established on 800 subjects, ages 6-adults. Test-retest reliability is excellent.

(3) Matching familiar figures (Kagan, 1966). This task is commonly used for assessing reflective versus impulsive reactions to stimuli. It will be administered and scored using the standard protocol. We will increase the sensitivity for subjects >11 years by timing the test for all subjects. Normative data is available from adults as well as our 60 women. Test-retest reliability is good at 0.85 and 0.77 for latency and number of errors, respectively.

MOTOR TASKS (DOMINANT AND NONDOMINANT HANDS)

It is important to determine dominant versus nondominant handedness because motor function has clear hemispheric lateralization. Handedness will be determined by a modification of the Crovitz method (Krovitz, 1962). Subjects are asked to demonstrate which hand they use for 8 activities. The hand chosen

for 6 or more activities is defined as the dominant hand. If one hand is chosen for 4 or 5 activities only, the woman is considered to be ambidextrous. The dominant foot is chosen from preference for 3 activities.
(1) The Pursuit Rotor (Chute, 1990). This task measures hand-eye coordination and requires the subject to utilize a computer mouse on a pad to control the movement of the graphic mouse on the computer screen. The goal is to keep the graphic "mouse" within a target circle that moves around a circular track at the rate of 35 mm/second (Macintosh version). The average time spent "off-target" and an average score derived from the distance traveled and the time off-target are calculated for both the dominant and nondominant hands. We have norms available from 200 women, ages 6-adults.
(2) Lafayette Pegboard (Klove, 1963). This motor test consists of subjects placing pegs in 25 round holes on a board, as quickly as possible. Both the dominant and nondominant hand are utilized. This test has wide usage. We have norms available from 200 women, ages 6-adults.
(3) Finger Tapping (Spreen et al., 1991). This test evaluates simple motor function and consists of a mechanical tapping key attached to a counter. Subjects tap with their index finger and are timed for five 10-second trials. Test-retest reliability is 0.58-0.93 (130). Norms are available from our own sample of 200 female subjects, ages 6-adults..
(4) Physical and Neurological Examination of Soft Signs (Paness), selected tests (Close, 1976). This test is a standardized, quantitative test of motor function. The dominant and nondominant hand and foot are tested individually. The time measurements include the time (seconds) required to complete twenty taps of index finger to thumb and to complete 20 taps by four sequential fingers to thumb. Similar timing is also performed for the dominant and nondominant foot tapping (heel remaining on floor). Norms are available from our own sample of 200 women, ages 6-adults.

SOCIAL FUNCTION (COMPETENCE)
(1) Symptoms Checklist-90 (Derogatis, 1977). This is a widely used self-report questionnaire identifies whether a subject is ‘bothered” by various psychiatric symptoms. It is short, widely used, and identifies 9 symptom dimensions and a global severity index.
(2) Young Adult Self-Report (20). The YASR is a self-report instrument designed for young adults age 18 to 30 years old. This questionnaire provides descriptions of the user’s behavior, thoughts, feelings, and competencies. It also provides information about several domains of the user’s life including friends, school, employment, and relationships. The normative sample for the YASR is comprised of subjects 18 to 27 years old drawn from a nationally representative sample.
(3) Beck Depression Inventory (Beck et al., 1996). This widely used 21 item self-report instrument was designed to measure the severity of depression in adults and adolescents 13 and older. The BDI-II was normed on a sample of college students as well as four different groups of psychiatric outpatients ages 13 to 86 years old.

EXECUTIVE FUNCTION
This category assesses the planning and sequencing of complex behaviors and attention span. It relies on frontal lobe function and subcortical connections. Executive function also includes the measures of Attention listed above.

(1) Wisconsin Card Sort Test (WCST; Chelune, 1985): The WCST is a neuropsychological test which measures abstract thinking, cognitive flexibility, categorizing, and nonverbal concept formation. It is particularly sensitive to frontal lobe abnormalities. The test consists of stimulus cards that differ in shape, color, and number that must be sorted. There is good normative data for children, adolescents, and adults as well as our own normative sample.

(2) The Tower of Hanoi (Reeves et al., 1994): This test measures the subject's capacity for planning and strategy and is sensitive to frontal lobe abnormalities. In the computerized version, the subjects sees three pegs and several disks of varying size on the computer screen. The task is to stack the disks in descending order on a specified peg. The subjects are required to transfer rings with certain permissible moves only, using a minimum number of moves (maximum of 25 moves). The total score is a function of accuracy, completion time, and problem difficulty. The time score is a function of the time spent and the maximum time allowed for the trial. Developmental changes in children 7 to 15 have been documented (109).We also have norms available from 200 normal females, ages 6-adults.

(3) Rey Osterrieth Complex Figure (Waber et al., 1985): The figure will be scored for the organizational component in both Turner subjects and controls, according to the method of Waber and Holmes.

(4) Verbal Fluencies: These tasks evaluate word finding ability and requires the child to name as many words as possible within a given category, within a specified period of time (1 minute). The Controlled Oral Word Association Test (phonological) assesses the number of words the subject can name beginning with the letters F, A, and S (Benton et al., 1989). The McCarthy scales semantic (McCarthy, 1972) assesses the number of words the subject can name in the categories: food, animals, clothing, and things to ride, 1 minute per category. Norms are available from 60 control adults in our own sample.

Academic achievement tests

WIDE Range Achievement Test-3 (WRAT3) (Wilkerson, 1993): Reading and arithmetic subtests. The WRAT3 is a brief achievement test. The reading subtest measures the ability to recognize and name letters and pronounce words. The Blue and Tan forms are normed for ages five through seventy four. Norms are available based a sample of 5000, starting with age five as well as our own normative sample. Test-retest reliability is adequate, ranging from 0.79 to 0.97.

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APPENDIX B- Psychosocial Evaluation

In collaboration with David Rubinow and his associates in the Behavioral Endocrinology Branch of the NIMH, we wish to assess the effects of Turner Syndrome on measures of mood, self-esteem, quality of life, social shyness and anxiety, and sexual function. Past and current psychiatric disorders will be evaluated by conducting a structured psychiatric diagnostic interview (SCID-IV) (1). Mood and behavioral symptoms will be monitored by administering visual analogue self-rating scales (2), as well as cross-sectional symptom rating scales consisting of the Hamilton Rating Scale for Depression (HRSD) (3) and the Center for Epidemiological Studies Depression Scale (CES-D) (4) (standardized rating scales measuring the severity of depressive symptoms completed by a rater (HRSD) or by the patient (CES-D), respectively). Additionally, we wish to monitor sexual function by employing Derogatis' Interview for Sexual Functioning (Female version) (DISF-SR) (5) a self report scale evaluating measures of sexual function including sexual activity and performance, as well as sexual fantasy and cognition. Finally, we wish to assess patients' quality of life, shyness and social anxiety, as well as self-esteem by using the following scales:

- Quality of Life Satisfaction Questionnaire (Q-LES-Q) (6) - A self-report measure evaluating the degree of enjoyment and satisfaction experienced by subjects in areas of daily functioning (e.g. work, leisure time, social relationships, physical health).
- Revised Shyness Scale (7) and the Social Interaction Anxiety Scale (SIAS) (8) - Brief self-report measures evaluating measures of shyness, social anxiety and avoidance.
- Rosenberg's self-esteem scale (9) - A brief scale evaluating dimensions of personal functioning related to the regulation of self-esteem.

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Appendix C Genetic counseling process analysis

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Introduction

Providing genetic counseling within a phenotype/genotype study of girls/women with Turner syndrome provides two important areas for genetic counseling research. One is an opportunity to record in a systematic way the concerns and issues faced by girls and women affected with Turner syndrome. This largely unstudied area will provide information about how to better meet the needs of these patients in a genetic counseling setting. It is our clinical impression that the major psychosocial issues facing these girls and women are social stigmatization due to short stature, dysmorphology and learning disabilities; infertility and other reproductive issues; decreased self-image: challenges in intimate relationships; and feelings of social isolation. These concerns are suggested by the small number of studies in the literature. One study of 59 adolescents with Turner syndrome identified peer ridicule and teasing to be predictive of depression and lower self-image (Rickert 1996). Another study of 22 middle-aged women with Turner syndrome identified infertility as their major psychosocial difficulty (Sylven 1993). Of 20 adult patients from Belgium, 10 expressed feelings of low self-confidence, depression and insecurity (Delooz 1993). A similar finding was reported among 30 adults with Turner syndrome who had an impaired self-esteem (McCauley 1986). However, there have been limited studies of the personal adjustment challenges faced by adolescents and women with Turner syndrome. This larger phenotype/genotype study provides an opportunity to explore and address further the concerns of this population.

Genetic counseling process analysis can also be done within the context of this larger study. Process analysis is the qualitative and/or quantitative evaluation of the interaction between provider and client. It has been used extensively in the fields of health communication and psychotherapy. Process research reveals what transpires in the professional exchange. Genetic counseling has been to date largely a clinical practice with few process or outcome studies (Marteau 1996, Bernhardt 2000, Biesecker 1999). The few significant studies were done by Dr. Lippman-Hand of prenatal genetic counseling conducted in the 1970s or by Dr. Michie to address the level of directiveness expressed by the counselor (Lippman-Hand 1979, Michie 1997). Kessler analyzed one transcript in the literature that demonstrated the utility of using both qualitative and quantitative approaches to analyzing counseling sessions in a manner comparable to the assessment of psychotherapeutic and medical

encounters (Kessler 1981, Richards 1996). Yet, there remain significant questions about how genetic counselors practice, what approaches they use, and how the process meets client needs (Kessler 1989 & 1992). Genetic counseling is a dynamic psychoeducational process with a great deal of practice variability. Process analysis will provide data on the goals of genetic counseling and how they are achieved, therapeutic interventions used by genetic counselors as well as unmet needs of clients. Models of counseling need to be designed from this empiric evidence to offer more consistency in practice and improved teaching methods.

One element of the genetic counseling interaction that may be studied quantitatively is the communication process. Dr. Debra Roter from The Johns Hopkins University has developed a quantitative assessment tool for health care communication, Roter Interaction Analysis System (RIAS), that has been used in a variety of health care professionals and settings (Hall 1988, Roter 1991 & 1997, Wissow 1994). It assesses what fraction of time the provider speaks compared to the patient, the balance between emotional and factual based information, the attention paid to the emotional concerns of the patient and to the patient's agenda. This tool codes each phrase or utterance into mutually exclusive and exhaustive categories. The categories include question-asking behaviors, exchange of bio-medical and psychosocial information, affective characteristics of the dialogue, and facilitation and orientation during the session. Application of RIAS to genetic counseling transcripts would provide a pilot analysis of the communication process in genetic counseling.

Further, a qualitative analysis of the transcripts, looking for the assessments made by genetic counselors, the strategies employed to address the concerns of the client and the responses by the clients would provide another source of data describing the process of genetic counseling. The larger phenotype/genotype study provides the opportunity to conduct this analysis more "purely" than in other genetic counseling settings. In this case, the clients are not likely to have significant educational needs, there is not an eminent decision facing the client (such as a prenatal or predictive genetic test), and the session is not the first time the client has heard the diagnosis of Turner syndrome. In such a setting, the focus of genetic counseling is likely to be the coping needs of the client, difficult aspects of living with Turner syndrome (quality of life), and overall adaptation. These tend to be the more therapeutic aspects of genetic counseling, which have infrequently been captured by process analysis. Outcomes of such a study would provide the basis for enhancing literature descriptions of genetic counseling, designing models of genetic counseling, teaching genetic counselors effectively, and for conducting follow-up research.

Study Objectives

- To identify major themes of interest and concern to women and girls affected with Turner syndrome
- To evaluate the communication process in genetic counseling in the context of counseling women and girls affected with Turner syndrome
- To identify therapeutic aspects of genetic counseling through process analysis
- To generate hypotheses in all three areas for further research

Study Design

These different analyses can all be done with a simply designed study that offers participants the opportunity to undergo genetic counseling for ninety minutes (or so) within their weeklong visit to the NIH. The genetic counseling sessions will be an invitation for the participants to discuss their concerns and any difficulties or struggles they face living with Turner syndrome. The process analysis involves audiotaping the sessions for verbatim transcription. The transcripts will be analyzed first looking for concerns spontaneously offered by the girls and women with Turner syndrome and those probed for by the counselor. A second qualitative analysis will be done looking for therapeutic techniques or strategies employed by the genetic counselor. Each of these processes will be augmented by the use of a qualitative software program called NUDIST. This program allows for systematic assignment of themes and note taking. It is estimated that 50 transcripts will be needed to saturate the data on themes discussed by the girls/women with Turner syndrome. It is possible that additional transcripts will be necessary in order to saturate the data for genetic counseling strategies since there may be greater variability in the styles and approaches of the counselor given the expressed needs of the participant. An interim analysis at 50 transcripts will determine how many are additionally needed. Subjecting the transcripts to computer analysis using the RIAS software program will result in a more analytical and complimentary analysis of the communication process. However, this quantitative data will be used in conjunction with the qualitative data to enhance its interpretation. Statistical significance is not relevant to the study unless a sufficient number of transcripts can be obtained and compared for specific quantitative dimensions. In this case, statistical consultation will be sought.

Eligibility

All participants 7 years old or older participating in the larger parent study will be invited to participate in a genetic counseling session with the explicit knowledge that to do so would involve an agreement to audiotaping and transcript analysis. Participants who do not agree to audiotaping will still be offered a clinical genetic counseling session as an adjunct to their participation in the phenotype/genotype study. Parents will be asked to allow their daughters to participate alone, however if the daughter requests that her parent(s) remain in the room, they will be invited to do so, but discouraged from actively participating in the dialogue.

Risks

Risks of the genetic counseling process analysis include discussion of emotionally difficult topics, such as self-esteem and identity issues, reproduction and issues of loss and adjustment. Genetic counseling will provide a forum to address these topics in a therapeutic manner. Yet, if the participant becomes unduly distressed or appears to have significant clinical depression (this will also be scored by the psychiatrists involved in the study), then the participant will be encouraged to agree to be seen by one of the psychiatrists involved in the phenotype/genotype study. They will also be referred to a psychotherapist or psychiatrist in their hometown for follow-up. Genetic counselors are trained to provide short term therapeutic support to clients who are mentally stable but facing difficult and stress-inducing life events (typically those related to a genetic condition).

Benefits

The nature of process analysis is such that the participant receives the equivalent of a clinical genetic counseling session. While the purpose is to study the process, by implication the process

ought to strive to effectively meet the client's needs. Thus, if one assumes that most clients benefit from clinical genetic counseling, then participants may view the genetic counseling session as a benefit to their participation in research. Since there is little empiric evidence that genetic counseling is effective in meeting client's needs, this remains an assumption. However, patient satisfaction studies suggest that clients are generally quite satisfied with genetic counseling services (Shiloh 1990, Michie 1997). A short follow-up counseling letter will be sent to participants as a supplement to the session. Participants may also construe this as a potential benefit of the process analysis.

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